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(54) **Eggs with balanced lipid composition**

(57) The present invention is related to an egg obtained from a domesticated bird, in particular a layer, having a lipid fraction balanced in seeds and green plant-type  $\omega 6$  and  $\omega 3$  fatty acids according to the ratio of seeds and green plant-type  $\omega 6$  fatty acids / seeds and green plant-type  $\omega 3$  fatty acids =  $1:1 \pm 10\%$  and having

a lipid fraction is balanced between polyunsaturated and saturated fatty acids according to the ratio of polyunsaturated / saturated fatty acids =  $1:1 \pm 10\%$ . The present invention is also related to a feed composition of exclusive vegetarian origin and suitable for poultry and a method for obtaining such egg from said poultry animals.

**Description****Field of the Invention**

[0001] The present invention relates to wild- or game-type eggs having an improved balanced lipid composition and which are compatible with modern recommendations to healthy dietary habits, and a method of feeding birds, in particular layers, that can be maintained under various rearing conditions (intensive, barn, plain air, free range, etc.) for the production of such eggs.

[0002] The significance of abbreviations used hereafter can be found in the part entitled "List of abbreviations".

**Background of the Invention and state of the art*****Evolution of human diet***

[0003] For many years, food available from vegetable and animal sources was low in fat (less than 25% of total energy intake), for it was of a wild type and because it was mostly eaten raw or grilled. It was source of about equal amount of saturated and polyunsaturated fatty acids as well as of  $\omega 6$  and  $\omega 3$  isomers ( $P:S = \omega 6:\omega 3 = 1:1$ ).

[0004] When rudimentary agriculture began to slowly bring changes in dietary habits, making food from animal origin (meat, fish, milk, eggs) more present in the daily diet, the contribution of cholesterol and saturated fatty acids to the total fat content proportionally increased. However, the  $\omega 6:\omega 3$  ratio remained relatively constant because traditional animal husbandry and fish culture did not widely differ in terms of feeding from former wild-type life, i.e. because the animals had large access to green-leaf vegetables (livestock) and phytoplanktons (fish).

[0005] Deviations from human food standards came readily into prominence at the turn of the century with the emergence of the modern agriculture and vegetable-oil industry.

[0006] Emphasis on  $\omega 6$ -rich grains fattening of domestic livestock and fishery and on partial selective dehydrogenation of  $\omega 3$ -rich vegetable oils results in a dramatic decrease in the amount of  $\omega 3$  fatty acids available to humans and to a not-less dramatic increase in the contribution of total fat and *trans* fatty acids to the daily energy supply of the human diet.

[0007] The drastic deviation operated some 150 years ago in human food habits changed the  $\omega 6:\omega 3$  ratio that was about 1:1 during the evolutionary period to a now estimated imbalance of about 10-11:1 for food from vegetable source and closer to 20-25:1 for mixed food from vegetable and animal sources. Meanwhile, the human genetic constitution remained relatively unchanged and did not cope with this brutal pace of change in the food chain.

Table 1

Time (years)				
-4.000.000	-10.000	1850	1950	2000
Total fat	25%	30%	35%	40%
P:S	1:1	0.9:1	0.75:1	0.5:1
$\omega 6:\omega 3$	1:1		10:1	20:1

[0008] Table 1 gives a scheme of the relative contribution of different dietary fatty acids (saturated fatty acids,  $\omega 6$  and  $\omega 3$  polyunsaturated fatty acids) to the human diet and possible changes subsequent to modern agriculture and industrial food processing, involving fattening of animal husbandry and hydrogenation of fatty acids.

[0009] In the meantime, wild animals, which were still left grazing on green leaves and wild plant seeds, continued to exhibit balanced essential fatty acid ratio's in their fat depots, independently from the species they belong to (Crawford, M.A. et al. (1989) "The food chain for n-6 and n-3 fatty acids with special reference to animal products", in Dietary  $\omega 3$  and  $\omega 6$  Fatty Acids - Biological Effects and Nutritional Essentiality (Galli, C. & Simopoulos, A.P., eds) NATO ASI Series A: Life Sciences Vol. 171, pp.5-19, Plenum Press, New York, ISBN 0-306-43231-5).

***Link with modern degenerative diseases***

[0010] Today,  $\omega 6$  and  $\omega 3$  PUFA are known to be essential in minimum amounts (see table 2 for adequate intake) for normal growth and development of humans. Their relative concentration or fractional ratio ( $\omega 6:\omega 3$ ) in food regulates the *in vivo* metabolism of lipoproteins, the fatty acid composition of cell membranes and the synthesis of some important biological mediators, the eicosanoids, which are essential to cell communication and global homeostasis. Scientific

evidences suggest that at least some human degenerative diseases have a food link and that the highly unbalanced modern dietary  $\omega 6:\omega 3$  ratio, as well as the strong contribution of total fats (more than 35% of total energy intake) and of saturated fatty acids ( $P:S = 0.5:1$ , including *trans* isomers) to the daily energy intake, may well have a direct implication into the appearance of some characteristic diseases of our modern society, i.e., cerebro- & cardiovascular diseases, coronary heart affections, cancers, diabetes, high blood pressure, chronic inflammatory and auto-immune diseases. More specifically, arachidonic acid, the  $\omega 6$  long-chain fatty acids derived from linoleic acid and/or obtained directly from food of animal origin, has been shown to effectively accumulate in modern man tissues when ingested in excess (A.T. Simopoulos (1991) Omega-3 fatty acids in health and disease and in growth and development, *Am. J. Clin. Nutr.* 54, 438-463). Consequently, arachidonic acid-derived eicosanoids have taken the lead in cell and tissue behaviour of modern man with all the deleterious consequences for health.

[0011] Scientific and epidemiological evidence seems to confirm that excess  $\omega 6$  fatty acids in modern man diet could well be one of the major culprit for reduced health performance in ageing.

#### The "balanced diet" notion

[0012] Galli & Simopoulos ("General recommendations on dietary fats for human consumption", in *Dietary  $\omega 3$  and  $\omega 6$  Fatty Acids Biological Effects and Nutritional Essentiality* (Galli, C. & Simopoulos, A.P., eds) pp. 403-404, North Atlantic Treaty Organisation Advanced Science Institute Series, Plenum Press, New York & London (1988)) defined a well-balanced diet as one that, among other things, provides max. 30% energy as fat together with a large proportion of monounsaturated fatty acids, an even distribution of saturated and polyunsaturated fatty acids ( $S:M:P = 1:6:1$ ), a ratio between  $\omega 6$  and  $\omega 3$  isomers not exceeding 5 to 1 ( $\omega 6:\omega 3 \leq 5:1$ ), and an optimal amount of 350-400 mg  $\omega 3$  long chain polyunsaturated fatty acids ( $\omega 3$  LC-PUFA).

[0013] In a recent workshop held at The Cloisters, National Institute of Health (NIH) in Bethesda, Maryland, USA, April 7-9, 1999, an Expert Panel led by Prof. A.T. Simopoulos established the following adequate intakes for  $\omega 6$  and  $\omega 3$  fatty acids in adults (table 2).

Table 2:

Adequate Intakes (AI) for Adults					
Fatty Acid	Abbrev	Formula	Grams/day *	Cal/da y	% total Cal.
Linoleic acid	LA	C18:2 $\omega 6$	4.44	40	2
$\alpha$ -linolenic acid	LnA	C18:3 $\omega 3$	2.22	20	1
EPA + DHA	$\omega 3$ LCP	C20/22:5/6 $\omega 3$	0.44	4	0.2

\*2000 kcal diet

[0014] It is seen that there is a clear trend towards re-adopting the historically-established, naturally-occurring lipid ratios settled by Nature 4 million years ago as those which must be optimally fit for human consumption. Quite remarkably, these modern recommendations for amounts and ratios of lipids in human diet are based on meta-analysis of epidemiological and clinical studies of the long term relationship between dietary habits and degenerative diseases in human.

[0015] Optimum dietary lipid pattern can be reached through substitution of  $\omega 6$ -rich for  $\omega 3$ -rich vegetable oils and enrichment of modern-type diets with greens, leaves and fish, but enrichment does *in fine* mean adding-up on fat.

[0016] Ideally, edible animal tissue lipids should also comply with these scientifically established evidence since pre-formed arachidonic acid from animal origin is known to incorporate very effectively in tissue lipids (Lands, W.E.M. (1997) Two faces of EFA, *Inform* 8, 1141-1147). Essentially, there is no scientific evidence for a need in dietary arachidonic acid in normal people. A very important aspect of wild-type animal tissue is that they favour  $\omega 3$  versus  $\omega 6$  long-chain phospholipids and contain substantially lower amount of arachidonic acid in comparison to their domesticated equivalent.

[0017] Scientifically and naturally established evidences suggest that healthy food from animal origins is of the wild or game-type, lean and balanced in essential fatty acids, low in arachidonic acid.

#### Eggs as food

[0018] Eggs are generally recognised as source of highly bio-available and valuable nutrients.

[0019] As a source of essential amino-acids, eggs are remarkable in that they are in perfect agreement with adult human needs; they are also a rich source of beneficial branched amino-acids (table 3).

Table 3:

Essential amino-acids in eggs and human needs		
Essential amino acids	mg / 100 g (two 60-g eggs)	**RDA (mg) 70-kg adult
*Isoleucine	682	840
*Leucine	1068	1120
Lysine	898	840
Cysteine + Methionine	680	700
Tyrosine + Phenylalanine	1174	1120
Threonine	600	560
Tryptophan	152	210
*Valine	762	980
<u>Lysine</u> <u>Cysteine + Methionine</u>	<b>1.22</b>	<b>1.20</b>

\*BCAA: branched chain amino-acids -

\*\*RDA: recommended dietary allowances (National Academy of Sciences, U.S.A., 1974)

[0020] Carbohydrates mainly appear in eggs as glucose and glycoproteins (table 4).

Table 4:

Carbohydrates in eggs			
	mg / 100 g (two 60-g eggs)		
	Albumen (68-g)	Yolk (32-g)	Total (100-g)
Free sugar*	260	260	520
Oligosaccharides**	330	110	440
Total	590	370	960

\*mainly D-glucose -

\*\* N- &amp; O-glycans in albumen, sialoglycans in yolk (Sugino, H., Nitoda, T. and Juneja, L.R. (1997) General Chemical Composition of Hen Eggs, In "Hen Eggs, Their Basic and Applied Science" (Yamamoto, T., Juneja, L.R., Hatta, H. &amp; Kim, M. eds) CRC Press Inc., Ch. 2, pp. 13 - 24).

[0021] Lipids in egg yolk appear as a mixture of neutral and polar lipids; their fatty acids are either long (C16-18) or very long (C20-22) carbon chains (table 5).

Table 5:

Lipids in eggs				
Lipid fractions	mg / 100 g (two 60-g eggs)			
	Tryglycérides	Phospholipids	Cholesterol	Total
Weight contribution	6,9	2,7	430	10
% fatty acid	96	72	~0	85.5
Fatty acids type	C16-C18	C16-C22	-	C18

[0022] Vitamins and minerals are omnipresent at relatively high concentration in eggs (table 6).

**Table 6 : Vitamins, minerals and oligoelements in eggs and human needs**

Vitamins		Name	RDI*	100g-egg	%RDI
Vit.A	a	Retinol	1,5150	0,1921	12,7
*Vit.D	b	Calciferol	0,0100	0,0012	12,0
Vit.E	c	Alpha-tocopherol	20,0000	1,4000	7,0
*Vit.K1		Phylloquinone			
Vit.B1		Thiamin	1,5000	0,0620	4,1
Vit.B2		Riboflavin	1,7000	0,5080	29,9
*Vit.B3 (PP)	d	Niacin	20,0000	0,0740	0,4
*Vit.B5		Panhotenic acid	10,0000	1,2540	12,5
Vit.B6		Pyridoxin	2,0000	0,1400	7,0
Vit.B8 (H)		Biotin	0,3000	0,0200	6,7
Vit.B9 (M)		Folic acid	0,4000	0,0460	11,5
Vit.B12		Cyanocobalamine	0,0060	0,0010	16,7
Vit.C	e	Ascorbic acid	60,0000		

Minerals		Name	2000 Cal	100g-egg	%RDI
Ca		Calcium	1000	50,0	5,0
P		Phosphorus	1000	178,0	17,8
Mg		Magnesium	400	10,0	2,5
Na		Sodium		126,0	
Cl		Chlorine		174,2	
K		Potassium		120,0	
S		Sulfur		164,0	
Oligo		Name	2000 Cal	100g-egg	%RDI
Fe		Iron	18,00	1,440	8,0
Zn		Zinc	15,00	1,100	7,3
Se		Selenium			
Cu		Copper	2,00	0,014	0,7
I		Iodine	0,15	0,048	32,0
Mn		Manganese		0,024	
Mo		Molybden			

All numbers in mg; 100-g eggs stands for the edible part of two 60-g-eggs

RDI : Reference Daily Intake = average values for healthy Americans over 4-y-old

RDI\* given for a 2000 Cal-diet

\*: accessory- or conditionally essential nutrients

a) retinol equivalent : 1 mg retinol or 3,3 IU or 2 mg b-caroten

b) calciferol: 1 mg or 40 IU

c) alpha-tocopherol equivalent (a-TE) : 1 mg d-a-tocopherol or 1.49 IU

d) niacin equivalent : 1mg nicotinic acid (or nicotinamide) or 60 mg tryptophan

e) ascorbic or dehydroascorbic acid

[0023] The weight-distribution of amino-acids, carbohydrates and lipids is genetically encoded in eggs whilst that of vitamins, minerals and fatty acids is sensitive to their concentration in the bird's diet.

[0024] The egg lipid composition has often been criticised in terms of its relatively high concentration in saturated fatty acids and cholesterol compared to total energy content. The scientifically-proven relationship (known as the "lipid hypothesis") between high CSI (cholesterol-saturated fat index) and relatively higher risk of cardio- and cerebrovascular diseases has often supported the critical evaluation of cholesterol content in food. Cell membranes of animal tissues differ from those of plant tissues in that they contain cholesterol and phospholipids bearing long chain polyunsaturated fatty acids. Egg yolk lipids conform to this rule: cholesterol and long-chain polyunsaturated fatty acids happen to appear in a ratio close to 1:1 (0.5 mM each in egg yolk) and the latter occur at the characteristic sn-2 position of tissue phospholipids. There is thus nothing special or "wrong" about the cholesterol level in egg - it just has to do with its primary function, i.e. to support the development of Life in avian. Cholesterol in egg yolk is almost a constant whatever the type of feed served to the chicken. However, the content of polyunsaturated fatty acids can be increased at the expenses of that of saturated and monounsaturated fatty acids by simple dietary means.

[0025] While amounts of saturated, mono-unsaturated,  $\omega 3$  and  $\omega 6$  polyunsaturated fatty acids have been separately varied in previous egg production and the effect of these minor changes on human blood lipid balanced were already described, eggs wherein all fatty acid and lipid fractions are controlled to result in a product which is compatible with a healthy diet defined as that one designed by Nature in the wild, were never produced nor described.

### Aims of the invention

[0026] The present invention aims to provide eggs obtained from domesticated birds, in particular layers, said eggs comprising an improved lipid balanced profile in order to result in a product compatible with healthy diet for humans and animals comparable to the one designed by nature in the wild.

[0027] Another aim of the present invention is to provide such safe and improved eggs and food compositions comprising such eggs that may be consumed as part of a balanced diet in reasonable quantity, as recommended by national and international health foundations, and that sustain consumer's health in the long term.

[0028] A further aim of the present invention is to provide a feed composition of exclusive vegetarian origin and a feeding method for poultry, in particular layers, from which said eggs having an improved lipid balanced profile may be obtained.

[0029] A last aim of the present invention is to provide such feed composition and feeding method for feeding poultry, in particular layers, that can be maintained in various conditions for the production of such eggs.

### Summary of the invention

[0030] The inventors have discovered that it is possible to obtain eggs from domesticated birds, preferably chicken eggs, having an advantageous lipid profile which complies with that of fat depots of wild- or game-type animals, that is balanced in saturated (30%) and polyunsaturated (30%) and is balanced in omega-6 (15%) and omega-3 (15%) fatty acids. Said eggs will be hereafter called wild-type or game-type eggs.

[0031] More precisely, the eggs according to the invention has a lipid fraction balanced in seeds and green plant-type  $\omega 6$  and  $\omega 3$  fatty acids according to the ratio of seeds and green plant-type  $\omega 6$  fatty acids / seeds and green plant-type  $\omega 3$  fatty acids =  $1:1 \pm 10\%$ .

[0032] The eggs according to the invention is also characterised in that their lipid fraction is balanced between polyunsaturated and saturated fatty acids according to the ratio of polyunsaturated / saturated fatty acids =  $1:1 \pm 10\%$ .

[0033] The eggs according to the invention may be consumed or incorporated in food compositions in reasonable quantities as part of a balanced diet, these food compositions sustaining consumer health in the long term. They may be used as a functional food or as medicament (see for reference Milner J.A., *Journal of Nutrition* (1999), Volume 129, Number 7S, "Functional foods and health promotion", pages 1395S-1397S).

[0034] The phospholipid fraction in said eggs is also characterised by an advantageous balanced fraction of animal-derived long chain fatty acids: omega-6 ( $\omega 6$ ) / omega-3 ( $\omega 3$ ) fatty acids equal to  $1:3 \pm 10\%$ .

[0035] Advantageously, the balanced lipid fraction of the eggs is made of green plant-type and animal-derived omega-3 ( $\omega 3$ ) fatty acids, characterised by the preferred following ratio: plant-type  $\omega 3$  fatty acids / animal-derived  $\omega 3$  fatty acids equal to  $5:1 \pm 10\%$ , a ratio similar to that recently proposed by the NIH Expert Panel for adequate intake of  $\omega 3$  fatty acids of plant and animal origins in human (table 2).

[0036] Preferably the eggs according to the invention have more than 450 mg/egg (about  $550 \pm 50$  mg/egg) of green plant-type fatty acids omega-3 ( $\omega 3$ ) and have more than 90 mg/egg (about  $110 \pm 10$  mg/egg) of animal-derived  $\omega 3$  fatty acids.

[0037] Advantageously, the eggs according to the invention contain no more than about 40 mg/egg, preferably about  $35 \pm 5$  mg/egg, animal-derived omega-6 ( $\omega 6$ ) fatty acids and is essentially arachidonic acid.

[0038] Advantageously, the animal-derived omega-3 ( $\omega$ 3) fatty acids contained in the eggs according to the invention are C20 and C22 fatty acids, preferably selected from the group consisting of eicosapentaenoic acid docosapentaenoic acid and docosahexaenoic acid.

[0039] Advantageously, said eggs contain about  $10 \pm 2$  mg eicosapentaenoic acid /egg, about  $15 \pm 3$  mg docosapentaenoic acid /egg and about  $75 \pm 15$  mg docosahexaenoic acid /egg.

[0040] Advantageously, the wild-type lipid profile of such eggs automatically results in an enrichment in vitamins, especially vitamin E, and naturally-occurring bacteriostatic enzymes such as lysozyme (about 36 mg/g dry matter albumin), in a reduction of about 10% of their cholesterol and saturated fatty acid content (Cholesterol-Saturated Fat Index, CSI index), the cholesterol content of said eggs being no more than 380 mg/% of the edible part.

[0041] The wild-type lipid profile of such eggs results also in the accretion of a substantial amount of animal-derived  $\omega$ 3 long chain polyunsaturated fatty acids (C20-C22) and in more than 50% reduction in arachidonic acid, comparatively to eggs available on the market.

[0042] Another aspect of the present invention is related to a feed composition of exclusive vegetarian origin in order to obtain from poultry, in particular layers, such eggs, said composition being a wild-type diet containing no animal fats and satisfying the equation of Huyghebaert et al. (Arch. Geflügelk (1995) 59(2), p.145-152) exposed hereafter and comprising 4 to 10% (w:w) of total fat, seed  $\omega$ 6 and green  $\omega$ 3 plant-type fatty acids contributing to total fat content in steadily decreasing manner from 40 to 15% and from 50 to 30%, respectively, and  $\omega$ 6: $\omega$ 3 essential fatty acid ratio being in favour of the green  $\omega$ 3 plant type fatty acids and decreasing from 0.8 to 0.5.

[0043] Preferably the feed composition according to the invention comprises about 30 to 40% carbohydrates, about 10 to 20% proteins, about 10 to 15% moisture, about 7 to 12% ash and about 4 to 10% fats, the total being 100%, for a total metabolisable energy of about 2800 kcal. The total comprises also addition of vitamin A, vitamin D3, vitamin E, methadione sodium bisulfite, riboflavine, panthothenic acid, niacine, vitamin B6, folic acid, biotin, thiamin, vitamin B12 and oligoelements (Mn, Zn, Fe, Cu, I, Se, Co, Ca), the preferred composition being the one described in the table 9.

[0044] The present invention is also related to a feeding method of poultry, in particular layers, comprising the step of feeding poultry with the feed composition according to the invention in order to readjust the  $\omega$ 6: $\omega$ 3 ratio in eggs, so that they present an improved balanced fatty acids composition according to the invention.

[0045] A last aspect of the present invention is related to a food composition comprising, as a food ingredient, the whole eggs, the eggs white or the eggs yolk of the eggs according to the invention, especially a food composition suitable for human consumption, including a functional food.

[0046] The present invention will be described in more details in the following detailed description of the invention and in the following examples.

### Detailed description of the invention

#### Designing the feed

[0047] The best feeding option would be the natural, wild-type one, whereupon the bird grazes on greens and leaves and collects insects and worms in a wild, "fight-or-flight" environment.

[0048] However, this method of production is not compatible with the growing need of an ever-expanding world population and economy. Greens are essentially source of  $\alpha$ -linolenic acid that is formed from linoleic acid in plant chloroplasts as a result of energy transfer from sunlight to chemical  $\pi$ -bonds. Given that the lipid fraction of green leaves account for about 1% of their total mass and that about 50% fatty acids therein are  $\alpha$ -linolenic acid, one can calculate that a normal 100-g portion leaves provides 0.5 g  $\alpha$ -linolenic acid. This is far from negligible when compared to the low concentration of this particular fatty acids in most edible seeds and oils, but total fat content ( $\pm 1\%$ ) is much too low to sustain a continuous process of egg production (an economically-viable way to produce healthy eggs at reasonable cost on a world-wide basis).

[0049] Within the group of green lipids, fits a unique exception from seeds in the name of flax seeds. Flax or linseeds are indeed an exceptional source of plant  $\omega$ 3 fatty acids otherwise found in much smaller amounts and higher  $\omega$ 6: $\omega$ 3 ratio in soya and canola seeds (table 7). Flaxseeds are also readily available at competitive world market price for feed ingredients. In this sense, flax seeds represent a unique source of green plant-type lipids which is contained within a seed in substantial quantities and at reasonable cost.

Table 7.

$\omega$ 3-containing greens, flaxseeds and Designer Feed (% of triglycerides)					
Vegetable Plant	SAFA	MUFA	PUFA		
	-	$\omega$ 7 + $\omega$ 9	$\omega$ 6	$\omega$ 3	$\omega$ 6: $\omega$ 3
Cabbage, red	25	5	30	40	0.75
Designer Feed	12	18	25	45	0.58
Parsley	18	3	26	54	0.48
Lettuce	18	3	17	44	0.38
Cabbage, white	18	8	15	58	0.26
Flax seeds	9	18	15	57	0.26
Cauliflower	22	15	13	50	0.26
Brussels sprouts	20	5	12	63	0.19
Spinach	12	3	8	52	0.16

[0050] Huygebaert (Arch. Geflügelk (1995) 59(2), p.145-152) has developed a mathematical model for the prediction of the fat composition in egg, in particular C16-C18, based on their respective contribution to total fat in the feed (table 8).

Table 8

Response in the egg yolk fat (%)						
y	Intercept	$x_1$	$x_2$	$(x_1)^2$	$(x_2)^2$	$x_1x_2$
C16:0	26.60	-1.462	0.191	0.0348	-0.0046	0.028
C18:0	7.94	-0.178	-0.121	0.0069	0.0029	0.010
C18:1	41.70	-2.637	0.378	0.0464	-0.0005	0.042
C18:2	-9.26	2.559	0.322	-0.1311	-0.0011	0.026
C18:3	-0.03	0.311	-0.016	-0.0202	0.0031	0.023
$x_1$ : dietary fat level-%; $x_2$ : the level-% of the respective characteristic in the dietary fat.						

[0051] Therefore, this models predicts for example that a concentration y of C16:0 fatty acids in the eggs can be obtained if the diet given to the poultry contains a concentration  $x_1$  of dietary fat and a concentration  $x_2$  of C16:0 fatty acids, the concentration y being calculated as follows:

$$y = 26.60 - 1.462x_1 + 0.191x_2 + 0.0348(x_1)^2 - 0.0046(x_2)^2 + 0.028x_1x_2$$

[0052] This model has been shown to be valid for the continuous production of wild-type eggs as defined, if the feed composition given to poultry comprises 4 to 10% (w:w) of total fat, seed  $\omega$ 6 and green  $\omega$ 3 plant-type fatty acids contributing to total fat content in steadily decreasing manner from 40 to 15% and from 50 to 30%, respectively, and  $\omega$ 6: $\omega$ 3 essential fatty acid ratio being in favour of the green  $\omega$ 3 plant type fatty acids and decreasing from 0.8 to 0.5. More precisely, the fat composition ( $\omega$ 6: $\omega$ 3 = Polyunsaturated:Saturated = 1:1) of the wild-type egg has been maintained constant for several months (> 18 months) under a defined feeding regimen.

[0053] Groups of 30,000 Isabrown birds were fed with a wild-type diet containing no animal fat, 35.5% carbohydrates, 17% protein, 12% moisture, 10.25% ash and 6.5% fat for a total of 2,800 Kcal metabolisable energy (M.E.). The detailed composition of the feed is given in Table 9.



**Table 9. Composition of Designed Feed**

5	Raw Material Inclusion	(%)	Analysis	(%)	
	Avizym 2300 - 20%	0.50	Protein	17.0	
10	Choline - 75%	0.05	Fat	6.5	
	Limestone gran.	8.29	Carbohydrate	35.5	
15	Layer supplement	0.25	Moisture	12.0	
	Salt	0.20	Ash	10.25	
	Dical. Phos.	1.26	M.E.	2,800 Kcal	
20	D,L-methionine - 40%	0.28			
	Course wheat	47.5			
	Course peas	10.0			
25	Wheat middlings	2.785		Total (%)	Digestible (%)
	Soya 50 / Hypro	15.33	Ca	3.7	-
30	Sunflower meal 30 profat	6.0	P	0.58	0.32
	Lucern 20 <sup>®</sup> (350)	2.53	Lys	0.89	0.74
35			Met	0.38	0.34
	Subtotal (DSF)	95.0	Cys+Met	0.68	0.57
			Thr	0.61	0.49
40	Vegetable oil (DM)	5.0	Trp	0.20	0.165
45	Total	100	Lys/ (Cys+Met)	1.30	

[0054] Layer supplement provides the following per kilogram of diet: vitamin A, 10,000 I.U.; vitamin D3, 2,000 I.U.; vitamin E, 10 I.U.; menadione sodium bisulfite, 0.6 mg; riboflavin, 5 mg; pantothenic acid, 10.9 mg; niacin, 40 mg; vitamin B6, 1 mg; folic acid, 0.5 mg; biotin, 20 µg; thiamine, 1 mg; vitamin B12, 20 µg; Mn, 75 mg; Zn, 55 mg; Fe, 35 mg; Cu, 7.5 mg; I, 1.9 mg; Se, 0.1 mg; Co, 0.7 mg, Ca, 330 mg; Mg, 55 mg.

[0055] Vegetable oil is cold-pressed flax seed oil (BS 6900: sediment, max. 0.25%; Iodine Value, min. 175; Acid Value, max. 4 mg KOH/g, Peroxide Value, max. 10, Colour Gardner, max. 13) stabilised with 0.4% Rendox (Kemin) containing BHA (E320), Ethoxyquin (E324), Citric Acid (E330), phosphoric acid (E338), mono- & diglycerides of fatty acids (E471), and enriched with 0.2% dl-α-tocopherol (Roche).

Designing the wild-type egg

[0056] The composition of fatty acids in egg yolk lipids can be modulated through dietary means. Especially, the  $\omega 3$  fatty acid of plant origin ( $\alpha$ -linolenic acid) can be incorporated at the expenses of saturated and monounsaturated fatty acids in the triglyceride fraction. Typically, regular eggs would contain almost undetectable level ( $< 1\%$ ) of  $\alpha$ -linolenic acid whilst free wandering birds grazing on greens and worms would almost incorporate 13% of  $\alpha$ -linolenic and show a balanced  $\omega 6:\omega 3$  ratio in the triglyceride fraction. Nutritionally, this change into the birds diet does not affect the way these lipids are metabolised in the body since  $\alpha$ -linolenic acid is usually burned and incorporated into tissues and cells membrane lipids at the same rate as monounsaturated fatty acids. A clear sign that this is indeed so is the fact that most  $\alpha$ -linolenic acid, when present in the yolk triglyceride fraction, is located at position sn-1/3, characteristic of non-essential fatty acids. However, it provides a unique back-up source of  $\omega 3$  fatty acids in fat depots for the synthesis of DHA through the fatty acids cascade pathway.

[0057] The major change associated with the presence of  $\alpha$ -linolenic in the wild birds diet is the ratio inversion of the animal derived long-chain polyunsaturated fatty acids in the phospholipid fraction of the yolk. Whilst regular feed contributes to the accretion of arachidonic acid at the expenses of docosahexaenoic acid (AA:DHA = 2:1), the wild-type feed favours the synthesis and deposition of docosahexaenoic acid (AA:DHA = 1:3).

Characteristics of wild-type eggs obtained with the designed feed*Fatty acid and lipid compositions of wild-type eggs compared to those of standard eggs*

[0058] After 3 weeks induction on Designer Feed, wild-type eggs can be followed for their fatty acid pattern. A validation study ran on five groups of hens during 19 months has allowed to establish a specification for the wild-type egg (table 10), as follows:

**Table 10. Fatty acid and lipid composition of wild-type eggs**

Fatty acids	% rel.	Lipids	% rel.
C16:0	19.34 $\pm$ 0.71	$\Sigma$ (SAFA)	28.5 $\pm$ 1.1
C18:0	9.18 $\pm$ 0.88	$\Sigma$ (MUFA)	40.9 $\pm$ 1.7
C16:1 $\omega$ 7	3.17 $\pm$ 0.42	$\Sigma$ (PUFA)	28.7 $\pm$ 1.6
C18:1 $\omega$ 9	37.74 $\pm$ 1.45	P/S	1.00 $\pm$ 0.08
C18:2 $\omega$ 6	13.59 $\pm$ 0.76	$\omega 6:\omega 3$	1.01 $\pm$ 0.07
C18:3 $\omega$ 3	11.69 $\pm$ 1.26	$\omega 6:\omega 3$ LCP	0.32 $\pm$ 0.03
C20:4 $\omega$ 6	0.81 $\pm$ 0.14		
C20:5 $\omega$ 3	0.28 $\pm$ 0.06		
C22:5 $\omega$ 3	0.43 $\pm$ 0.10		
C22:6 $\omega$ 3	1.86 $\pm$ 0.39	*C20:4 $\omega$ 6	35 $\pm$ 5 mg

\*Content of arachidonic acid (AA, C20:4 $\omega$ 6) calculated as %rel. x 4,200 mg fatty acids per 50-g egg edible portion.

[0059] During the same period, standard eggs available on the market and obtained from Europe, the United States,

South and East Asia were collected and analysed according to the same procedure for their fatty acid and lipid contents. Results show that standard eggs substantially deviate from wild-type egg (table 11). In particular, standard eggs are extremely poor (max. 1%) in plant-type  $\omega$ 3 fatty acids and, as a consequence, they are also much richer in arachidonic acid.

**Table 11. Fatty acid and lipid composition of standard eggs**

Fatty acids	% rel.	Lipids	% rel.
C16:0	22.63 $\pm$ 2.32	$\Sigma$ (SAFA)	31.0 $\pm$ 2.5
C18:0	8.37 $\pm$ 0.75	$\Sigma$ (MUFA)	44.8 $\pm$ 3.7
C16:1 $\omega$ 7	3.26 $\pm$ 0.89	$\Sigma$ (PUFA)	20.9 $\pm$ 4.6
C18:1 $\omega$ 9	41.5 $\pm$ 3.30	P/S	0.68 $\pm$ 0.18
C18:2 $\omega$ 6	17.03 $\pm$ 4.06	$\omega$ 6: $\omega$ 3	12.03 $\pm$ 5.0
C18:3 $\omega$ 3	0.66 $\pm$ 0.37	$\omega$ 6: $\omega$ 3 LCP	2.06 $\pm$ 1.0
C20:4 $\omega$ 6	2.03 $\pm$ 0.30		
C20:5 $\omega$ 3	0.01 - 0.02		
C22:5 $\omega$ 3	0.13 $\pm$ 0.05		
C22:6 $\omega$ 3	1.03 $\pm$ 0.40	*C20:4 $\omega$ 6	85 $\pm$ 15

\*Content of arachidonic acid (AA, C20:4 $\omega$ 6) calculated as %rel.  $\times$  4,200 mg fatty acids per 50-g egg edible portion.

[0060] Other eggs, often declared as  $\omega$ 3 docosahexaenoic acid enriched, were analysed in detail for their fatty acids distribution. These eggs are normally obtained from hens fed with a docosahexaenoic acid oil-enriched feed (fish oil, algae, single cell oil, etc.) and their content of this specific fatty acid is relatively higher than in other eggs. It also turned out that such eggs exhibit a fatty acid composition reminiscent to that of standard eggs in terms of their low content in wild-type plant  $\omega$ 3 fatty acids (max. 1%) and their high content in domestic-type animal-derived  $\omega$ 6 long-chain fatty acids, notwithstanding the additional fact that, when animal-derived  $\omega$ 3 fatty acids were summed up, they had less of these than the wild-type egg obtained with an exclusive vegetarian diet.

#### *Stability of wild-type eggs compared to that of standard eggs*

[0061] Wild-type eggs, naturally rich in  $\omega$ 3 fatty acids, may be assumed less stable than modern  $\omega$ 6-rich eggs. Nutritionists and consumers may raise more specific concerns towards fatty acids and cholesterol peroxidation in wild-type eggs.

#### *a. Stability with hen's age*

[0062] Fatty acid changes with hen's age. The influence of hen's age on  $\omega$ 3 long-chain phospholipids content in wild-type eggs was followed on the life-cycle of two independent groups of 30,000 birds each. The data show that although a very slight trend down is observed, the effect of age on fatty acid composition in wild-type eggs is minor (table 12).

**Table 12. Content of  $\omega$ 3 long-chain phospholipids in wild-type egg as a function of hen's age.**

Group 1		Group 2	
Week	$\Sigma \omega 3$ LCP (%)	Week	$\Sigma \omega 3$ LCP (%)
38	2.62	26	2.59
43	2.49	31	2.77
46	2.62	34	2.75
49	2.53	44	2.35
52	2.45	48	2.41
56	2.31	56	2.67
60	2.28	61	2.45
70	2.53	$m \pm \sigma$	$2.57 \pm 0.15$
$m \pm \sigma$	$2.48 \pm 0.15$		

$\Sigma \omega 3$  LCP (%) as sum of EPA + DPA + DHA

**b. Stability with egg's age**

[0063] Fatty acid changes with egg's age. Wild-type eggs were analysed for their fatty acid composition (from Fatty Acid Methyl Ester (FAME) spectrum analysis) 3 and 9 weeks after being laid and stored at room temperature (21°C). After 9 weeks, the yolk were barely separable from the white. The most obvious change upon storage is in the level of docosahexaenoic acids (-9% / 3 weeks, -18% / 6 weeks and -27% / 9 weeks at 21°C). All other fatty acids were kept at a remarkable constant level (table 13).

**Table 13. Changes in fatty acid composition of wild-type egg with age**

Fatty acids	% rel.		
	Fresh	3-w	9-w
C16:0	18.48	18.72	18.50
C18:0	8.72	8.16	8.45
C16:1 $\omega$ 7	3.29	3.58	3.38
C18:1 $\omega$ 9	39.96	40.05	39.66
C18:2 $\omega$ 6	13.17	13.60	14.19
C18:3 $\omega$ 3	11.00	11.10	11.14
C20:4 $\omega$ 6	0.74	0.77	0.79
C20:5 $\omega$ 3	0.25	0.22	0.24
C22:5 $\omega$ 3	0.42	0.31	0.33
C22:6 $\omega$ 3	1.86	1.69	1.36

  

Lipids	% rel.		
	Fresh	3-w	9-w
$\Sigma$ (SAFA)	27.2	26.9	26.9
$\Sigma$ (MUFA)	43.2	43.6	43.0
$\Sigma$ (PUFA)	27.4	27.7	28.0
P/S	1.01	1.03	1.04
$\omega$ 6: $\omega$ 3	1.03	1.08	1.15
$\omega$ 6: $\omega$ 3 LCP	0.29	0.35	0.41
*C20:4 $\omega$ 6	31.1	32.3	33.2

\*Content of arachidonic acid (AA, C20:4 $\omega$ 6) calculated as %rel.  $\times$  4,200 mg fatty acids per 50-g egg edible portion. Ref. Anal. Malvoz 97-05-05

*c. Stability with processing temperatures*

[0064] Fatty acid changes with processing temperatures. Both wild-type and standard eggs were tested for their stability against oxidation during typical culinary practices, i.e., boiling (hard eggs) and baking (cake and baked custard) (tables 14 & 15) and compared.

**Table 14.**

Changes in fatty acid composition of wild-type egg upon cooking				
Fatty acids	% rel.			
	Fresh	Boiled	Cake	Custard
C16:0	18.48	18.34	17.97	18.81
C18:0	8.72	8.49	8.48	8.78
C16:1 $\omega$ 7	3.29	3.17	2.00	3.04
C18:1 $\omega$ 9	39.96	37.9	37.00	37.68
C18:2 $\omega$ 6	13.17	14.1	16.56	14.68
C18:3 $\omega$ 3	11.00	12.64	12.20	11.48
C20:4 $\omega$ 6	0.74	0.74	0.71	0.71
C20:5 $\omega$ 3	0.25	0.26	0.21	0.18
C22:5 $\omega$ 3	0.42	0.44	0.42	0.42
C22:6 $\omega$ 3	1.86	1.74	1.76	1.83

Table 15.

Changes in fatty acid composition of standard egg upon cooking				
Fatty acids	% rel.			
	Fresh	Boiled	Cake	Custard
C16:0	22.51	-	22.64	22.97
C18:0	8.11	-	7.63	8.57
C16:1 $\omega$ 7	3.46	-	3.27	3.52
C18:1 $\omega$ 9	40.32	-	40.13	41.74
C18:2 $\omega$ 6	17.84	-	19.87	15.74
C18:3 $\omega$ 3	0.79	-	1.48	0.69
C20:4 $\omega$ 6	2.18	-	1.80	1.90
C20:5 $\omega$ 3	0.01	-	0.03	0.02
C22:5 $\omega$ 3	0.17	-	0.13	0.13
C22:6 $\omega$ 3	1.16	-	1.02	0.97

[0065] These results show that the fatty acid composition of fresh and cooked wild-type and standard-type eggs are identical within the limit of accuracy.

#### d. Setting upon boiling

[0066] The rates of setting of the eggs upon boiling were also compared between standard and wild-type eggs. Eggs were bored in order to have a hole in the shell at the apical side where the air chamber lies (without perforing it) and were boiled for various time (8 to 12 min.) in hot water (min. 400 ml per egg). At the end of the incubation, eggs were rapidly cooled in a large volume of cold water and stored one night in the fridge. The day after, the eggs were peeled and sliced in twice. The appearance of the yolk in standard and wild-type eggs was compared. No difference in rate of setting could be assigned at any time between the two types of eggs.

#### Cholesterol content in wild-type eggs compared to standard eggs

[0067] Wild-type and standard eggs were boiled, cooled, dried and peeled. The white was separated from the yolk and the cholesterol content in the yolk was determined. Based on the weight of the different fractions (intact egg, shell & membranes, albumen, yolk), the amount of cholesterol in 100-g egg edible portion of wild-type and standard eggs was calculated (table 16).

Table 16.

Cholesterol in wild-type and standard eggs				
	Standard eggs		Wild-type eggs	
	Egg N°1	Egg N°2	Egg N°1	Egg N°2
Total weight (g)	62.83	62.74	66.40	63.45
Shell & membranes (g)	7.45	6.13	7.45	6.60
Albumen (g)	37.10	39.49	39.77	39.92
Yolk (g)	18.22	17.09	19.12	16.88
Cholesterol/yolk (%)	1.394	1.245	1.197	1.262
Cholesterol/egg (mg/%)	459	376	388	375

[0068] Whilst the cholesterol content of standard eggs is about  $410 \pm 10$  mg/egg (mg/%), the cholesterol content of wild-type eggs is about  $410 \pm 10$  mg/egg (mg/%) of the edible part. In other words, the cholesterol of wild-type eggs

according to the invention is reduced by about 10% as compared to the one of standard-type eggs.

#### *Vitamin E content in wild-type eggs compared to standard eggs*

[0069] Among the antioxidants present in eggs, vitamin E plays an essential role in stabilising the lipids against oxidation and rancidity. Wild-type eggs, richer in sensitive  $\omega 3$  fatty acids, are advantageously enriched with vitamin E in order to avoid peroxidation of cholesterol and other lipid fractions. Designer feed is enriched with 0.2% dl- $\alpha$ -tocopherol acetate in order to maintain 10 mg vitamin E per 50-g edible egg (table 17).

Table 17.

Vitamin E content (mg/%) in wild-type eggs compared to standard-type eggs						
	Standard	Wild-type				
Egg tested	Egg N°1	Egg N°2	Egg N°3	Egg N°4	Egg N°5	Average *
$\alpha$ -toco pherol	6.2	18.7	23.9	19.8	19.3	20.4 $\pm$ 0.2
$\gamma$ -toco pherol	2.1	2.03	1.9	2.15	2.3	2.1 $\pm$ 0.2

\*(mg/%) a 50-g egg edible portion contains min. 10 mg vitamin E.

#### *Lysozyme content in wild-type eggs compared to standard eggs*

[0070] The effect of changing dietary lipids on hen's capacity to produce essential active enzymes and proteins for the protection of eggs against invasion by pathogens was tested by measuring the amount of lysozyme present in wild-type egg as compared to that in standard eggs (table 18).

Table 18.

Lysozyme content in Standard and Wild-type eggs		
Lysozyme	Standard type	Wild type
mg/g dry matter albumen	31.3 $\pm$ 4.3	36.0 $\pm$ 2.0

[0071] As seen, the wild-type egg contains 15% more lysozyme in average than standard eggs. The reason for this is not known at this stage, but it probably has a positive influence on the resistance of wild-type eggs in a jungle-like environment.

#### Nutritional make-up of the wild-type egg to human

[0072] Egg lipids are made of fat store lipids (triglycerides, TG) and structural lipids (phospholipids, PL and cholesterol, CHL). These occur in egg yolk in a constant specific ratio (TG:PL:CHL = 16:6:1). Most fatty acids are concentrated in the triglyceride and the phospholipid fractions whilst cholesterol is almost totally (90-95%) unesterified. Fatty acids in the two fractions are not randomly distributed: essential fatty acids are mostly present at position sn-2 of the triglyceride and the phospholipid fractions whilst non-essential fatty acids (FA) occur at position sn-1/3 of the triglyceride fraction and at position sn-1 of the phospholipid fraction.

[0073] The distribution of fatty acids in egg yolk lipids drives their postprandial influence on blood lipids. In the digestive tract, they are hydrolysed by pancreatic 1,3-lipase and 2-phospholipase to free fatty acids, sn-1,3 monoglycerides and sn-2 lysophospholipids, respectively. Their reconstitution in the intestinal enterocytes results into the formation of triglycerides bearing essential fatty acids at position sn-2 and, among others, long-chain polyunsaturated fatty acids at position sn-1,3. Triglycerides with essential fatty acids at position sn-2 are known to have hypocholesterolaemic effect in human whilst long-chain polyunsaturated fatty acids at position sn-1,3 of blood triglycerides are directly available for tissue incorporation through their release by endothelial 1,3-lipase. The bioavailability of egg yolk long-chain polyunsaturated fatty acids is similar to that of those from other animal tissues and must be very similar to that of endogenously produced long-chain polyunsaturated fatty acids.

#### Prophylactic effects of egg lipids

[0074] Given that egg yolk long-chain polyunsaturated fatty acids are extremely bio-available for incorporation into tissue- and circulating cells membrane lipids and that their  $\omega 6:\omega 3$  ratio is susceptible to changes through dietary means,

it is interesting to assess the influence of the bird's diet on the healthiness of the eggs destined to human consumption.

[0075] Egg lipids contain small amounts (less than 20% of total fatty acids content) of short and medium chain (C12-16) fatty acids. Their location at position sn-1/3 of TG and sn-1 of PL makes them available for direct energy production or for storage in adipose tissue. Monounsaturated fatty acids and polyunsaturated fatty acids present at position sn-2 of triglycerides contribute to the hypocholesterolaemic effect of egg lipids. Long-chain polyunsaturated fatty acids located at position sn-2 of PL's are available for tissue incorporation.

[0076] Seen as a food lipid vector, egg is ranking high in the range of dietary fats (dairy and meat produces, vegetable and fish oils). Fish oil is often reckoned as a good source of  $\omega 3$  long-chain polyunsaturated fatty acids. However, two-third of long-chain polyunsaturated fatty acids in fish oil are associated with the sn-2 position of the TG which makes them less bio-available and more susceptible to be diluted in fat depots and thus more prone to oxidative deterioration. It has been recommended to take large amount of vitamin E supplements together with fish oil.

[0077] As compared to regular eggs that are almost totally depleted of the wild-type plant linolenic acid (linolenic acid (LnA) < 1%; ratio linoleic acid:  $\alpha$ -linolenic acid (LA:LnA) > 30:1), wild-type eggs supply these two essential fatty acids in a balanced ratio (LA:LnA = 1:1) and contribute to the endogenous synthesis of long-chain polyunsaturated fatty acids via the fatty acids biological cascade in the liver. The absence of  $\alpha$ -linolenic acid in the regular diet of layers also leads to the preferential accretion of arachidonic acid in egg yolk phospholipids ( $\omega 6:\omega 3$  LC-PUFA = 2:1) whilst this ratio is inverted in wild-type eggs ( $\omega 6:\omega 3$  LC-PUFA = 1:3). Direct incorporation of dietary long-chain polyunsaturated fatty acids in tissues and circulating cells is thus in favour of  $\omega 3$  long-chain polyunsaturated fatty acids with the wild-type eggs.

[0078] It is known that the effect of  $\omega 3$  fatty acids on serum cholesterol concentration is similar to those of other unsaturated fatty acids (monounsaturated and  $\omega 6$  polyunsaturated), i.e., when they replace C12-16 saturated fatty acids at the sn-2 position of triglycerides, they lower serum cholesterol.  $\omega 3$  long-chain polyunsaturated fatty acids have an added benefit of consistently lowering serum triglyceride concentration through reduction of chylomicron and VLDL secretion by the intestine and the liver, respectively.

[0079] When fed to selected groups of people, the eggs according to the invention have been shown to indeed contribute to the improvement of: (a) circulating cell membranes fatty acid composition ( $\omega 3:\omega 6$  long-chain polyunsaturated fatty acids ratio), (b) blood lipid distribution (no statistical change in blood cholesterol level, improved distribution within blood lipoproteins - HDL/LDL balance, substantial reduction in the amount of fat circulating in the blood), (c) blood pressure (5 to 10% reduction in both systolic and diastolic pressure) and, even (d) breast milk lipid composition (60% and 300% increase in  $\alpha$ -linolenic acid and docosahexaenoic acid, respectively, with no substantial changes in other fatty acids).

[0080] Finally, through this feeding practice of the chicken, it has been feasible to readjust the  $\omega 6:\omega 3$  ratio in eggs so that they present a balanced fatty acid composition comparable to the original "wild-type food" available to early man. As a lipid source, the egg according to the invention thus belongs to the minor family of  $\omega 3$ -rich fats and oils and lies in between those from vegetable and fresh water fish origins (Table 19).

Table 19.

$\omega 3$ -containing seeds, fish oils compared to wild-type egg					
(% of triglycerides)					
Vegetable/fish lipid source	SAFA	MUFA	PUFA		
	-	$\omega 7 + \omega 9$	$\omega 6$	$\omega 3$	$\omega 6:\omega 3$
Wheat germ	20	18	55	7	8
Soybean	16	22	54	7.5	7
Walnut	11	15	62	12	5
Canola	7	63	20	10	2
Egg according to the invention	30	40	13	13	1
Salmon	20	30	5	5	1
Trout	25	30	6	6	1

[0081] The eggs according to the invention and river fish deliver a minimum of 70% unsaturated fatty acids (the healthy one), equal amounts of both  $\omega 6$  and  $\omega 3$  polyunsaturated fatty acids ( $\omega 6:\omega 3 = 1:1$ ) and substantial amounts of animal-derived  $\omega 3$  long-chain polyunsaturated fatty acids in a favourable ratio ( $\omega 6:\omega 3 = 1:3$ ) (table 20).



Table 20.

$\omega$ 3 LC-PUFA in the egg according to the invention and river fish		
$\omega$ 6: $\omega$ 3	PUFA	LC-PUFA
Egg according to the invention	1.03	0.35
Salmon	0.98	0.32
Trout	0.92	0.20

[0082] Furthermore, the eggs according to the invention are also characterised by advantageous organoleptic properties in terms of freshness and flavour. They are rich in vitamins and antioxidants and are produced from layers effectively maintained immunised against *Salmonella* infections through the diet according to the invention they receive, said diet being rich in oligosaccharides naturally present in green and leaves.

[0083] Moreover, the eggs according to the invention can be defined as being "kosher" for layers, because they are maintained in a diet for poultry naturally rich in vitamin K, which prevents the occurrence of blood spots on the yolk vitellin membrane and provides an additional barrier to eventual *Salmonella* transovarian infections of the yolk.

#### LIST OF ABBREVIATIONS

[0084]

P: polyunsaturated fatty acid

S: saturated fatty acid

M: monounsaturated fatty acid

AA: arachidonic acid

EFA: essential fatty acid

FA: fatty acid

LC-MUFA: long-chain monounsaturated fatty acid

LC-PUFA: long-chain polyunsaturated fatty acid

LCP: long-chain polyunsaturated fatty acid

PUFA: polyunsaturated fatty acid

MUFA: monounsaturated fatty acid

SAFA: saturated fatty acid

EPA: eicosapentaenoic acid

DHA: docosahexaenoic acid

DPA: docosapentaenoic acid

LnA:  $\alpha$ -linolenic acid

LA: linoleic acid

CHL: cholesterol

CSI: cholesterol-saturated fat index

TG: triglyceride

PL: phospholipid

VLDL: very low density lipoprotein

HDL: high density lipoprotein

LDL: low density lipoprotein

$\omega$ 3 fatty acids: fatty acids having whith first insaturation on carbon 3 from the terminal methyl, as known by the man skilled in the art

$\omega$ 6 fatty acids: fatty acids having whith first insaturation on carbon 6 from the terminal methyl, as known by the man skilled in the art

#### Claims

1. An egg obtained from a domesticated bird, in particular a layer, having a lipid fraction balanced in seeds and green plant-type  $\omega$ 6 and  $\omega$ 3 fatty acids according to the ratio of seeds and green plant-type  $\omega$ 6 fatty acids / seeds and green plant-type  $\omega$ 3 fatty acids = 1:1  $\pm$  10% and having a lipid fraction balanced between polyunsaturated and

saturated fatty acids according to the ratio of polyunsaturated / saturated fatty acids =  $1:1 \pm 10\%$ .

2. The egg according to claim 1, **characterised in that** its phospholipid fraction is balanced according to the ratio of animal-derived  $\omega 6$  fatty acids / animal-derived  $\omega 3$  fatty acids =  $1:3 \pm 10\%$ .
3. The egg according to claim 1 or claim 2, balanced in plant-type and animal-derived  $\omega 3$  fatty acids according to the ratio of plant  $\omega 3$  fatty acids / animal  $\omega 3$  fatty acids =  $5:1 \pm 10\%$ .
4. An egg according to claim 1, comprising green plant-type fatty acids ( $\omega 3$ ) in a concentration comprised between 450 and 600 mg/egg.
5. The egg according to any one of the preceding claims, whose animal-derived  $\omega 3$  fatty acids account for more than 90 mg/egg and whose composition comprises C20 & C22  $\omega 3$  fatty acids, the C20 and C22 fatty acids being preferably selected from the group consisting of eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid.
6. The egg according to any one of the preceding claims, which contains no more than 40 mg/egg of animal-derived  $\omega 6$  fatty acids, and which is essentially arachidonic acid.
7. The egg according to any one of the preceding claims, which contains no more than 380 mg cholesterol /% of the edible part.
8. Feed composition of exclusive vegetarian origin for poultry, in particular layers, able to produce the egg according to any one of the preceding claims, **characterised in that** it satisfies the mathematical model of Huygebaert (Huygebaert (Arch. Geflügelk (1995) 59(2), p.145-152)), said composition containing no animal fat and comprising as ingredients 4 to 10% (w:w) of total fat, seed  $\omega 6$  and green  $\omega 3$  plant-type fatty acids contributing to total fat content in steadily decreasing manner, 40 to 15% (w:w) and from 50 to 30% (w:w), respectively, and  $\omega 6:\omega 3$  essential fatty acid ratio being in favour of the green  $\omega 3$  plant type fatty acids and decreasing from 0.8 to 0.5, the total (w:w) of the ingredients of the composition being 100%.
9. Method for obtaining the egg according to any of the claims 1 to 7 from poultry, comprising the step of feeding said poultry with the composition according to claim 8.
10. A food composition comprising, as a food ingredient, the whole egg, the egg's white or the egg's yolk of the egg according to any one of the claims 1 to 7, in particular a food composition suitable for human consumption.



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 00 87 0109

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	EDER K.: "Laying performance and fatty acid composition of egg yolk lipids of hens fed diets with various amounts of ground or whole flaxseed" ARCHIV FÜR GEFLÜGELKUNDE, vol. 62, no. 5, 1998, pages 223-238, XP000956233 * the whole document *	1-10	A23L1/32 A23L1/30 A23K1/18 A23K1/14
X	DE 43 14 899 A (WOOBANG LAND CO) 9 December 1993 (1993-12-09) * page 2, line 3 - page 4, line 53; claims 1-8, 10 *	1-10	
X	CHERIAN G.: "Omega-3 fatty acid and cholesterol content of newly hatched chicks from alpha-linolenic acid enriched eggs" LIPIDS, vol. 27, no. 9, 1992, pages 706-710, XP000956185 * abstract; tables * * page 709, column 1 - page 710 *	1-10	
X	AN B.: "Effects of dietary fat sources containing omega3 or omega6 polyunsaturated fatty acids on fatty acid composition of egg yolk in laying hens" KOREAN JOURNAL OF ANIMAL SCIENCE, vol. 41, no. 3, 1999, pages 293-310, XP000956227 * the whole document *	1-10	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			A23L A23K
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>24 October 2000</b>	Examiner <b>Boddaert, P</b>
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EPO FORM 1503 02/02 (Pv/Cv1)



European Patent  
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# EUROPEAN SEARCH REPORT

Application Number  
EP 00 87 0109

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Place of search THE HAGUE		Date of completion of the search 24 October 2000	Examiner Boddaert, P
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons Δ : member of the same patent family, corresponding document</p>			

EPO FORM 1508 03/82 (P0401)



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Application Number  
EP 00 87 0109

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**ANNEX TO THE EUROPEAN SEARCH REPORT  
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